

Claims

What is claimed is:

1. A method of detecting the presence or absence of a target body in a specimen, the method comprising

5 obtaining a specimen field exposed to or labeled with at least a first fluorophore and a second fluorophore, the first fluorophore emitting photons at a first wavelength and the second fluorophore emitting photons at a second wavelength;

exposing the specimen field to light sufficient to excite the first and second fluorophores;

10 scanning the specimen field at a low magnification for first sources of photons at the first wavelength and for second sources of photons at the second wavelength;

registering the location of each first source and each second source within the specimen field;

acquiring and recording a first image of the specimen field at each location,

15 the first image generated via an optical or electronic filter that substantially blocks photons of the second wavelength but is permissive for photons of the first wavelength;

acquiring and recording a second image of the specimen field at each location at a high magnification, the second image generated via an optical or electronic filter that substantially blocks photons of the first wavelength but is permissive for photons of the

20 second wavelength;

indexing each first image and each second image to the corresponding location within the specimen field; and

inspecting a first image and second image at a single location within the specimen field,

25 wherein the presence of a candidate body in the first and second images at the single location indicates the presence of a target body in the specimen.

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2. The method of claim 1, wherein preparation of the specimen field comprises:

- a. lysing the cell sample to give a sample mixture;
- b. centrifuging the sample mixture;
- c. separating the supernatant from the sample mixture;
- d. resuspending the resulting pellet of cells in a physiological buffer solution;
- e. plating the cells on an adhesive slide;
- f. adding cell culture media to the slide.

3. The method of claim 2, wherein preparation of the specimen field further comprises:

after step d, making a dilution of the cell mixture, treating the dilution with a dye sensitive for dead cells, and performing a cell count to determine the sample cell density for the slide to be used.

4. The method of claim 2, wherein the target body is a cancer, epithelial, smooth muscle, dendritic, memory T-, memory B-, somatic, normal, aberrant, or stem cell.

5. The method of claim 2, wherein the system is capable of detecting at least one target cell in a specimen field of at least 1,000,000 cells.

6. The method of claim 2, wherein the recoding comprises at least a 1024 x 1024 pixel array image

7. The method of claim 2, wherein the field specimen comprises white blood cells as the majority of cell types.

8. A detection system comprising

a stage for receiving a specimen field;

a detector positioned and configured to acquire images of locations within the specimen field at a set level and one or more additional amplifications of the set level;

a light source positioned and configured to expose the specimen field to light sufficient to excite a first fluorophore at a first excitation wavelength and sufficient to excite a second fluorophore at a second excitation wavelength;

a camera attached to the detector, the camera positioned and configured to (1) capture a first image at a location in the specimen field via an optical or electronic filter that substantially blocks photons at a second emission wavelength of the second fluorophore but is permissive for photons at a first emission wavelength of the first fluorophore, and (2) capture a second image at the location in the specimen field via an optical or electronic filter that substantially blocks photons at the first emission wavelength but is permissive for photons at the second emission wavelength; and

a computer that records the first image and second image and indexes the first image and second image to the corresponding location within the specimen field, the computer displaying, on demand by a user, the first image and second image for the corresponding location.

9. A method for analyzing for biological agent cells in a specimen field of cells comprising:

- i) treating the specimen field with a first fluorophore that identifies the biological agent cell;
- ii) treating the specimen field with a second fluorophore that identifies the biological agent cell;
- iii) exposing the specimen field with light suitable for causing the first fluorophore to emit photons,
- iv) exposing the specimen field with light suitable for causing the second fluorophore to emit photons,
- v) identifying cells in the specimen field that are emitting photons, which cells are biological agent cells.

10. The method of claim 9, wherein the specimen field cell preparation comprises:

- i. centrifuging a sample mixture;
- j. resuspending the sample mixture;
- 5 k. plating the cells on an adhesive slide;
- l. treating the slide with paraformaldehyde;
- m. treating the slide with Triton;
- n. treating the slide with a pre-hybridization solution;
- o. treating the slide with a hybridization solution having a fluorophore;
- 10 p. treating the slide with a fluorescent dye.

11. The method of claim 10, further comprising treating the specimen field with one or more additional fluorophore(s) that identifies the biological agent cell and exposing the specimen field with light suitable for causing the one or more additional fluorophore(s) to emit photons.

12. The method of claim 11, wherein at least one fluorophore identifies DNA of a biological agent cell.

13. The method of claim 10, wherein the biological agent is bacteria, Rickettsiae, viruses, fungi, or prions.

14. The method of claim 1, wherein preparation of the specimen field comprises:

- a. lysing the blood sample with ammonium chloride solution;
- b. centrifuging the sample mixture;
- c. separating the supernatant ammonium chloride solution and erythrocytes;
- d. resuspending the resulting pellet of white cells in PBS;
- 30 e. centrifuging the sample mixture;
- f. resuspending the resulting pellet of white cells in PBS;

- g. making a dilution of the cell mixture of step f, tryphan blue, and PBS;
- h. plating the cells on an adhesive slide;
- i. adding cell culture media to the slide.

5 15. A method for screening a transplantation organ donor for the presence or absence of a target body comprising the method of claim 2, wherein the specimen field is a sample taken from the organ donor.

10 16. A method for assessing the efficacy of a drug candidate against a disease or disease symptom in a subject who was administered the drug candidate by screening for the presence or absence of a target body whose presence or absence is indicative of the disease or disease symptom comprising the method of claim 2, wherein the specimen field is a sample taken from the subject.

15 17. A method for screening a blood sample for the presence or absence of a target body comprising the method of claim 2, wherein the specimen field is a blood sample.

20 18. A method for screening a fluid sample for the presence or absence of a target body comprising the method of claim 2, wherein the specimen field is a fluid sample.

25 19. The method of any of claims 15-18, wherein the target body is a cancer cell.

 20. A method of screening for the presence of bacteria comprising the method of claim 2, wherein at least one fluorophore comprises a DNA probe for bacteria.

30 21. The method of claim 20, wherein the specimen field is taken from a surgical patient after surgery.

22. The method of claim 20, wherein the specimen field is taken from a food sample.

23. The method of claim 10, further comprising:

- j. exposing the slide to an aldehyde-based fixative;
- k. rinsing the slide in phosphate-buffered saline (PBS);
- l. adding human AB serum to the slide;
- m. adding a primary antibody to the slide and incubating the slide;
- n. rinsing the slide in PBS;
- o. adding a secondary antibody to the slide and incubating the slide;
- p. exposing the slide in an organic solvent;
- q. rinsing the slide in PBS;
- r. adding human AB serum to the slide;
- s. adding a primary antibody to the slide and incubating the slide;
- t. rinsing the slide in PBS;
- u. adding a secondary antibody to the slide and incubating the slide;
- v. rinsing the slide in PBS;
- w. adding a cell dye to the slide and incubating the slide;
- x. rinsing the slide with PBS;
- y. exposing the slide to water;
- z. mounting the slide.

24. The method of claim 10, further comprising;

- j. exposing the slide in an organic solvent;
- k. rinsing the slide in PBS;
- l. adding a primary antibody to the slide and incubating the slide;
- m. rinsing the slide in PBS;
- n. adding a secondary antibody to the slide and incubating the slide;
- o. rinsing the slide in PBS;
- p. adding a cell dye to the slide and incubating the slide;
- q. rinsing the slide with PBS;

- r. exposing the slide to water;
- s. mounting the slide.

25. The method of any of claims 23 or 24, wherein the organic solvent is an
5 alcohol or acetone.

26. The method of any of claims 23 or 24, wherein the primary antibody is
keratin.

10 27. The method of any of claims 23 or 24, wherein the secondary antibody is
anti-rabbit rhodamine.

28. The method of claim 23, wherein the primary antibody in step s is keratin
and the secondary antibody in step u is anti-rabbit rhodamine.

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